Metabolism

فريق طوفان الأقصى

Modified N: 21

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Nucleotide Metabolism

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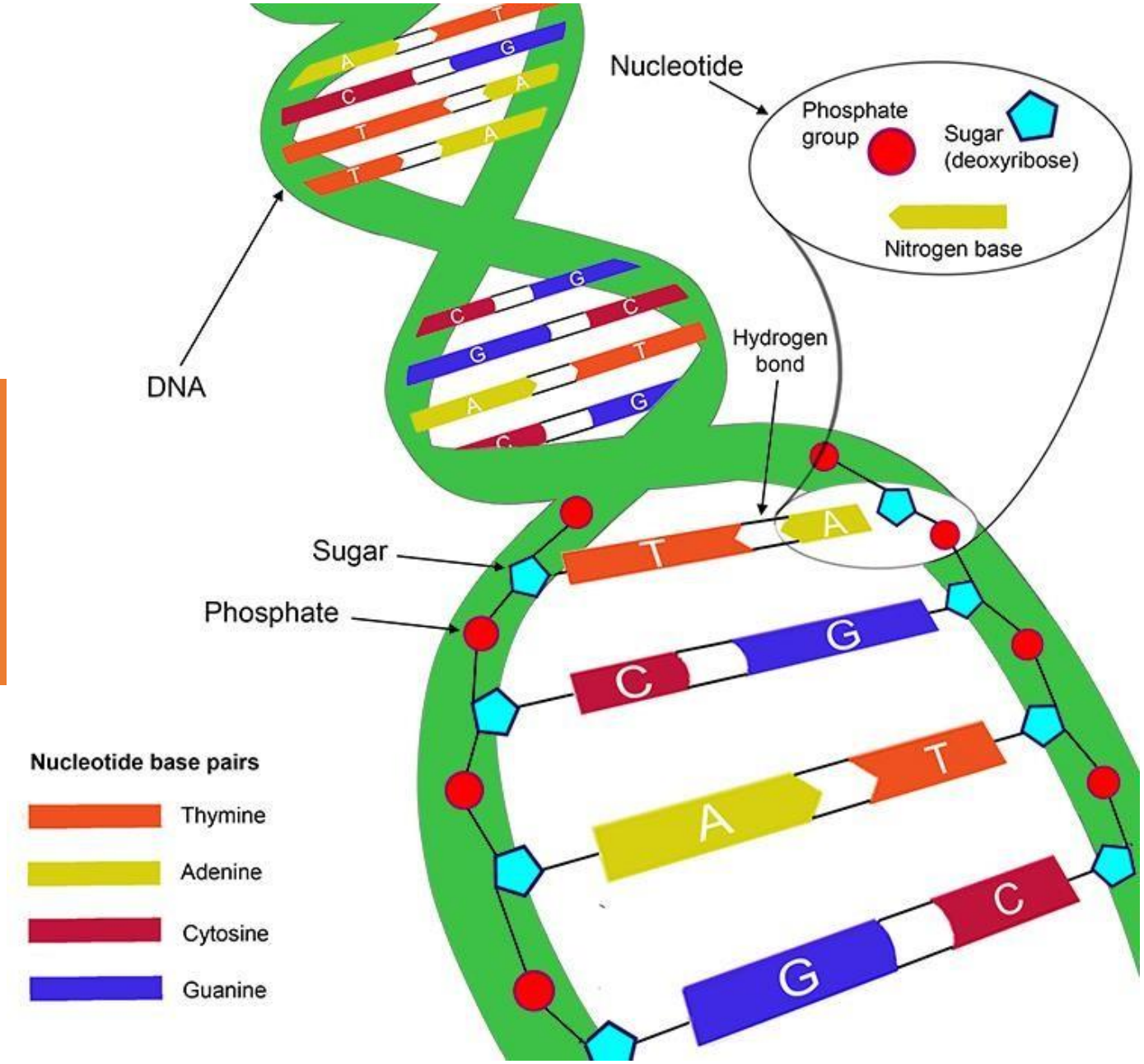
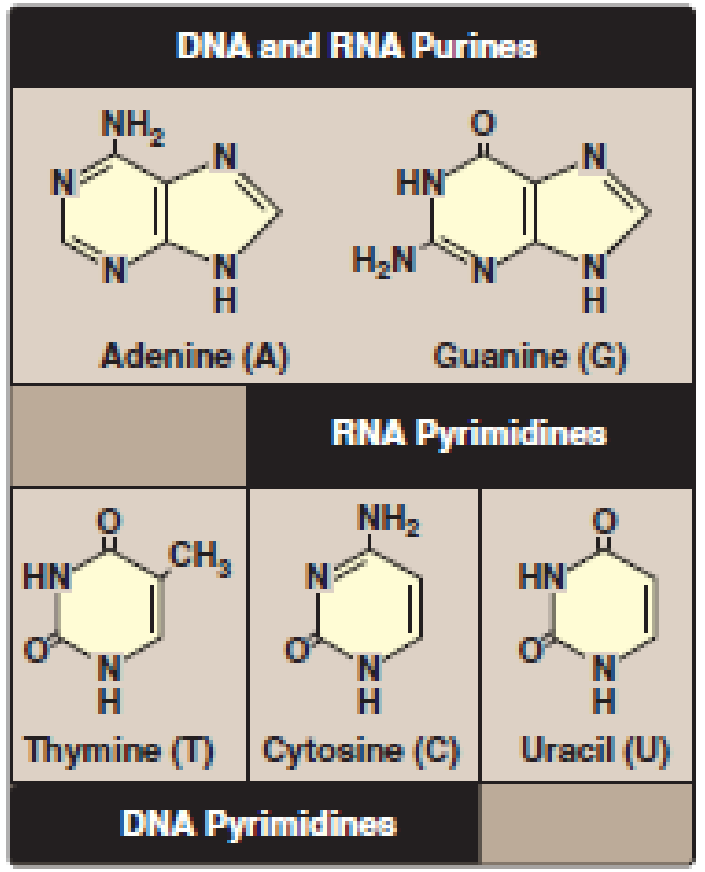


All images were taken from Lippincott's Biochemistry textbook except where noted

(mammothmemory.net)

Purine and pyrimidine structures and roles

- Essential for RNA and DNA synthesis.
- **Nucliotides:Nitrogen containing compounds in their nitrogenous bases and they have multiple functions**
- They serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins, such as, UDP-glucose and CDP-choline **(glycogen synthesis, bilirubin conjugation)**
- They are structural components of several essential coenzymes, such as coenzyme A, FAD, NAD+, and NADP+.
- They serve as second messengers in signal transduction pathways, such as cAMP and cGMP
- They are "energy currency" in the cell. **Like ATP,GTP...)**
- They act as regulatory compounds for many metabolic pathways by inhibiting or activating key enzymes.

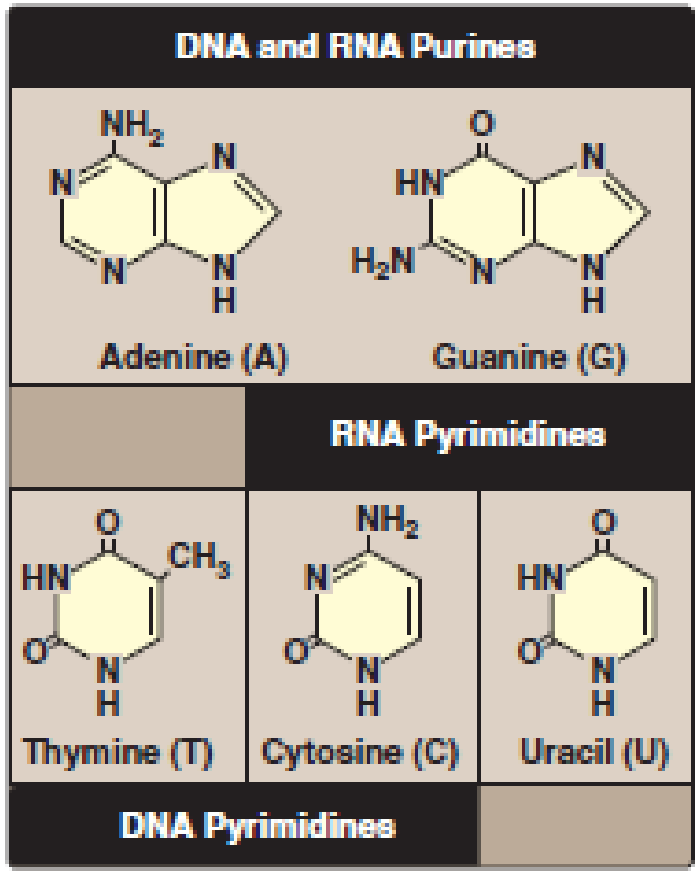


LIKE ENZYMES THAT GET ACTIVATED OR INHIBITED BY THE BINDING OF ATP,GTP.....

- **The complement in this slide:**
- Nucleotides can be classified based on their nitrogenous bases into 2 types **Purines** and **Pyrimidines**.
- The only part that is different between nucleotides.

ممكن تربط الي اسمه قصير الشكل تاعه كبير

- **Purines** consist of two fused rings —a **5-membered ring** and a **6-membered ring**— while **pyrimidines** consist of a single **6-membered ring**
- They contain nitrogen in their structure--> **nitrogen containing compounds.**
- Purines--> A,G Pyrimidines-->C,U,T CUT ✂



Nucleosides

Nucleoside= Pentose sugar + Base

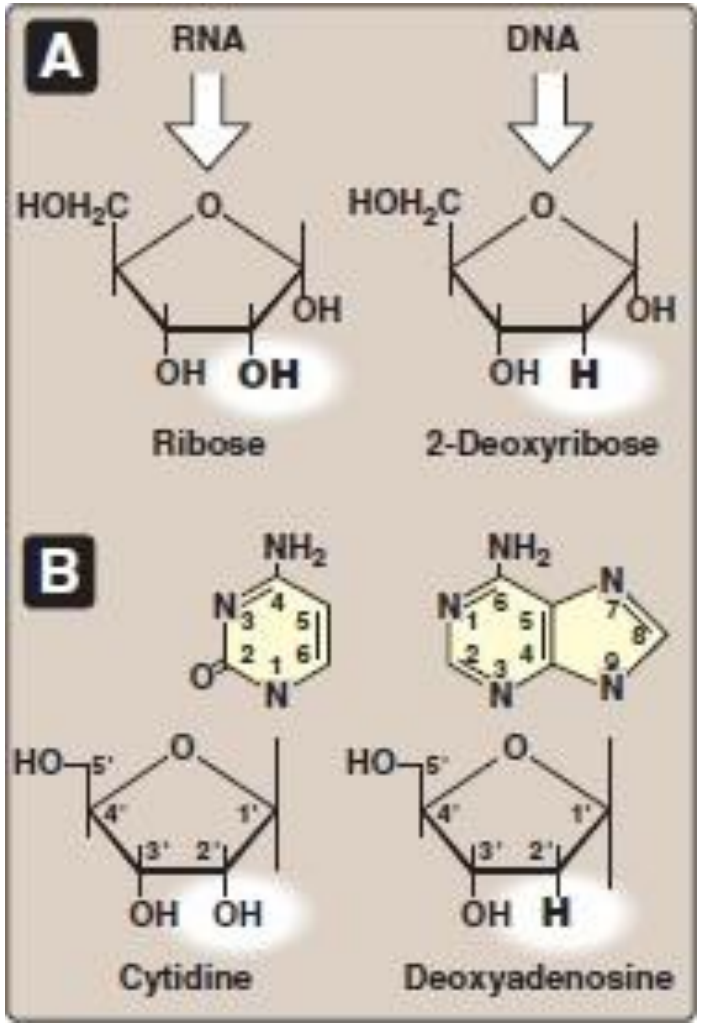
Ribose + base = Ribonucleoside

The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively.

2-deoxyribose + base = deoxyribonucleoside.

The deoxyribonucleosides of A, G, C, and T are named deoxyadenosine, deoxyguanosine, deoxycytidine, and deoxythymidine, respectively.

Numbering is separate and different (prime and on prime)



Nucleotides

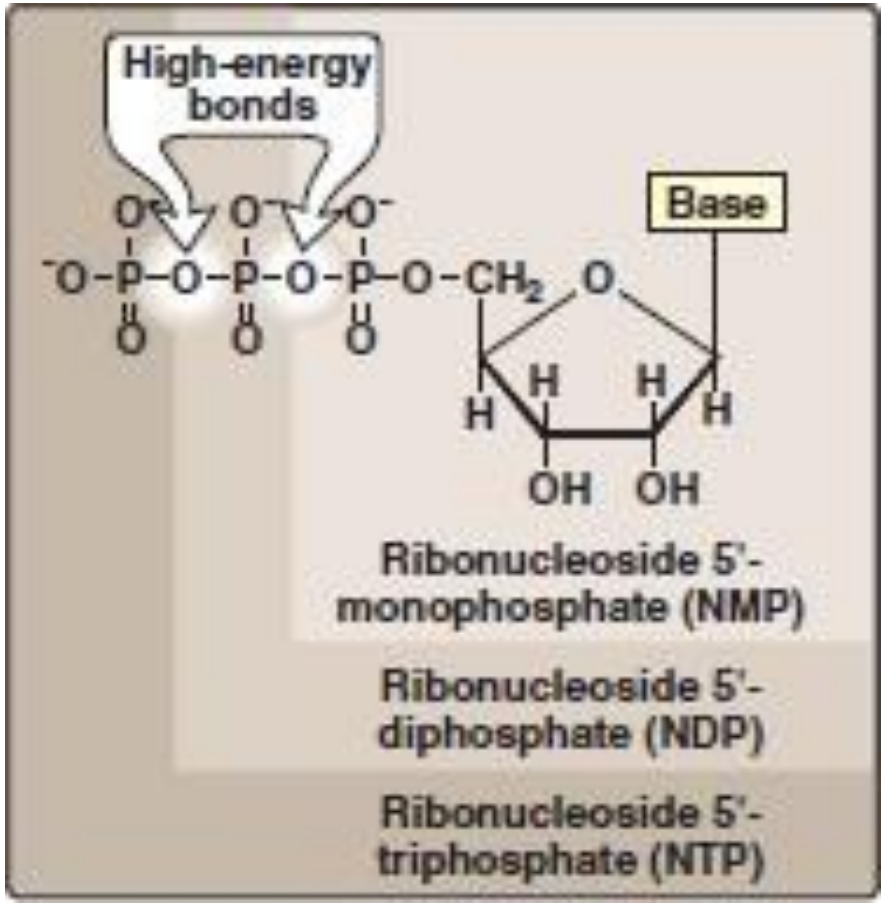
Nucleoside + one or more phosphate groups= Nucleotide

The first P group is attached by an ester linkage to the 5'-OH of the pentose forming a nucleoside 5'-phosphate or a 5'-nucleotide. **When I say nucleotide, I can't tell how many phosphates are there.**

The type of pentose is denoted by the prefix in the names "5'-ribonucleotide" and "5'-deoxyribonucleotide."

The second and third phosphates are each connected to the nucleotide by a "high-energy" bond. **That's why we use them as energy currency.**

The phosphate groups are negatively charged causing DNA and RNA to be nucleic acids.



- **The complement in this slide:**
- The nitrogenous base is attached to carbon number 1 of the sugar whether this sugar is ribose or deoxyribose.
- Phosphates are attached on carbon number 5

- ATP--> Adenosine triphosphate

Sine mean nucleoside and as we said before nucleoside + phosphate = nucleotide

Mono--> AMP Di--> ADP Tri--> ATP

So now is ATP nucleoside or nucleotide? yes, Nucleotide

Remember:

Ribonucleoside 5'-monophsphate = Ribonucleotide

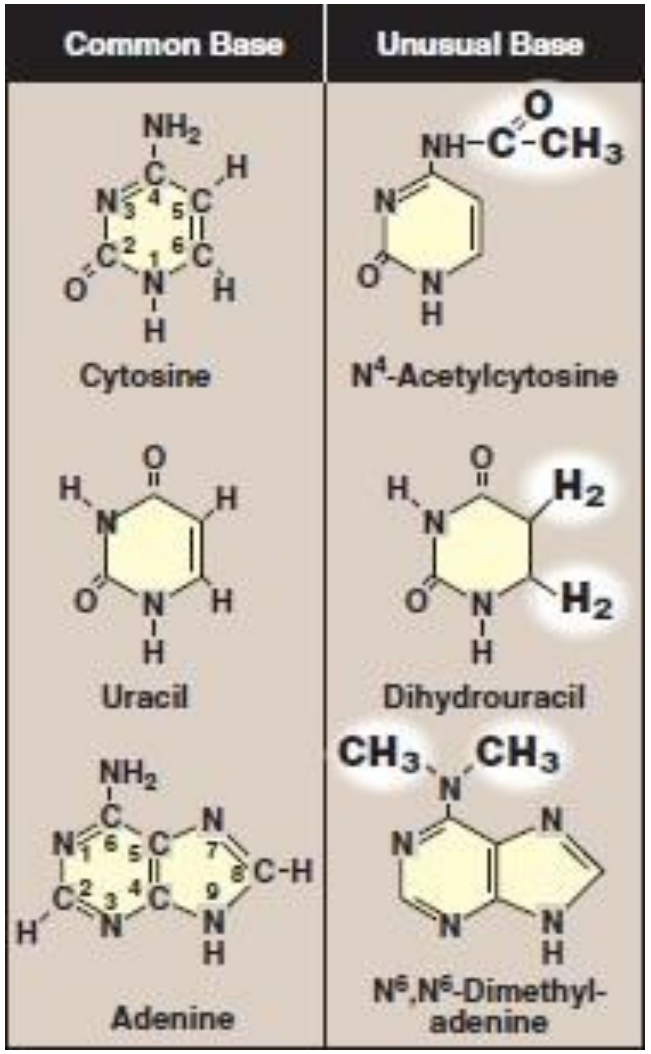
Ribonucleoside 5'-diphsphate = Ribonucleotide

Ribonucleoside 5'-triphsphate = Ribonucleotide

So, I can't know the number of phosphates that are attached to nucleoside when I say nucleotide.

Base modifications

- **NOTE:** these nitrogenous bases can be modified by covalent modifications for different purposes .
- Can occur also for proteins, and it is one of the changes that occurs for regulation, during aging....



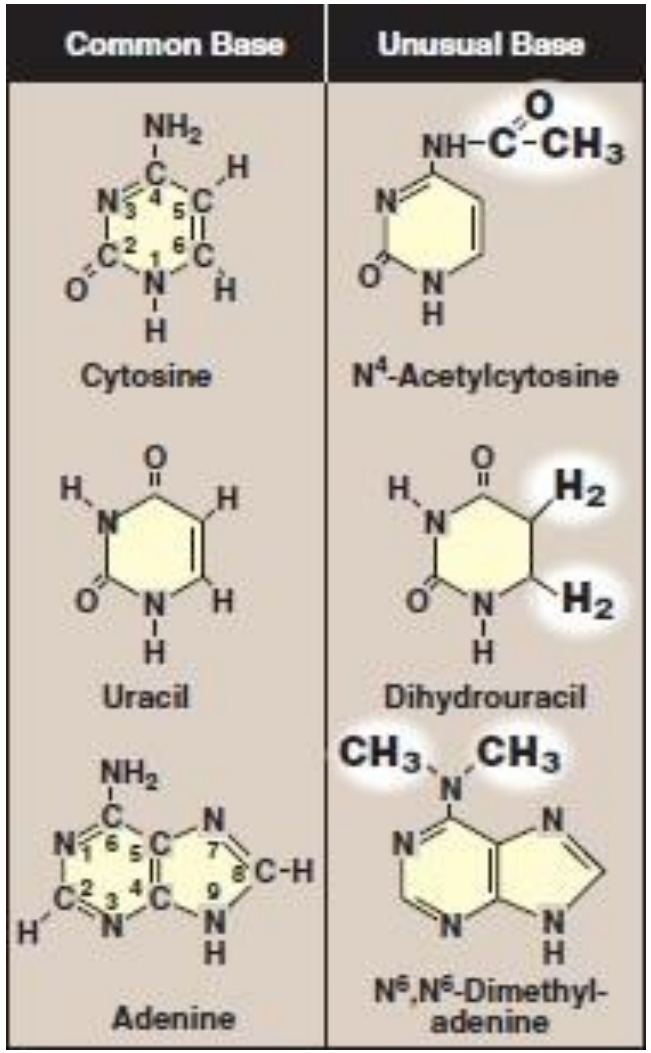
Acetylation

Reduction

Methylation

Or glycosylation

The presence of an unusual base in a nucleotide sequence may aid in its recognition by specific enzymes, or protect it from being degraded by nucleases



- **The complement in this slide:**
- Acetylation can occur, for example, for cytosine and mostly activates gene expression, making it easier for transcription factors to attach to the gene, resulting in more gene expression.

- Reduction can happen to uracil for different purposes in the cell.

- Methylation results in gene silencing, affecting gene expression. That's why, in some cells, the synthesis of specific gene products occurs, while in other cells, it doesn't because of covalent modification including methylation.
  For example X chromosome inactivation happens by methylation.

Doctor said that we learned about it in molecular biology, did we? Idk xD

- Glycosylation results in addition of sugar molecule.

Purine and pyrimidine synthesis

Sugars and phosphates can come from PPP(pentose phosphate pathway Lec 3) and here we will focus on Purine and pyrimidine synthesis.

- The purine and pyrimidine bases can be synthesized de novo

I can get purine and pyrimidine either from diet or synthesis them, there are 2 ways to synthesis them, the first one: de novo: from scratch, so I'm going to gather all these atoms in nitrogenous bases, and we will see how AAs are involved as sources of nitrogen.

- Or can be obtained through salvage(like recycle, shorter-->max 2 steps) pathways (reuse of the preformed bases resulting from normal cell turnover).

الدكتورة شبهتهم بعمل البيتزا
بالطريقة الاولى de novo انا بدي اجيب طحين وبيض وزيت وابصر شو واعمل عجينة بعدين بيتزا فراح اطول
بالطريقة الثانية salvage انا بشتري البيتزا مجمدة او عجينة جاهزة فعسريع بتجهز) مش هكتب مثال السيارة اسف(😔😒

- Little of the purines and pyrimidines supplied by diet are utilized, and are degraded instead

de novo is not a major source.

Purine synthesis- the contributing compounds

The atoms of the purine ring are contributed by a number of compounds:

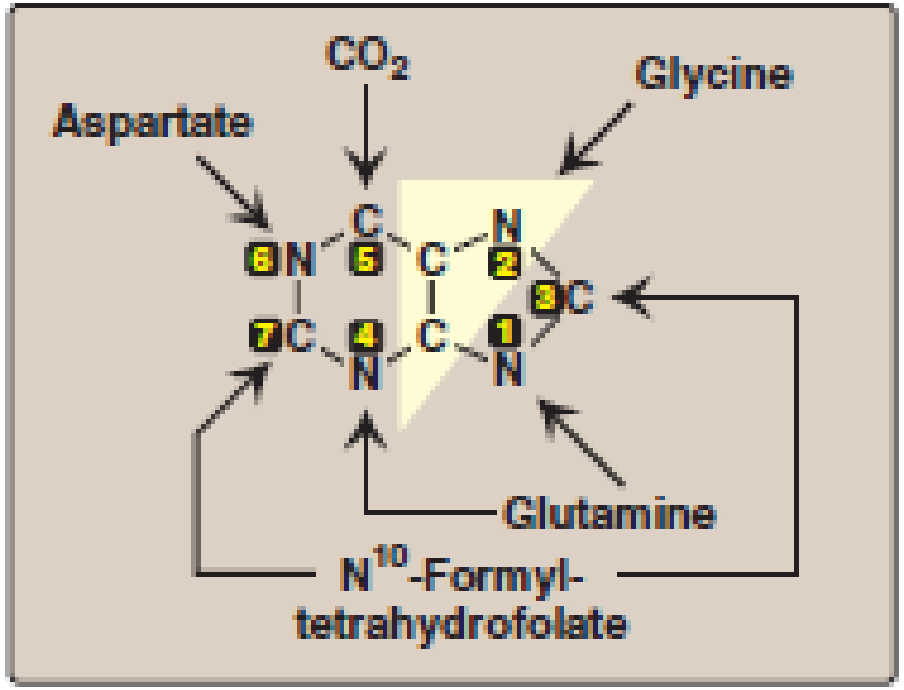
1. Amino acids (aspartic acid, glycine, and glutamine)
   all nitrogens are coming from AAs
2. CO2 --> giving carbon

3. N10–formyltetrahydrofolate --> giving C=O (formyl group)

The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate.

Ribose 5-phosphate is synthesized by the pentose phosphate pathway

NOTE: read the first point the the next slide before this slide. Important!!!!!



Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes

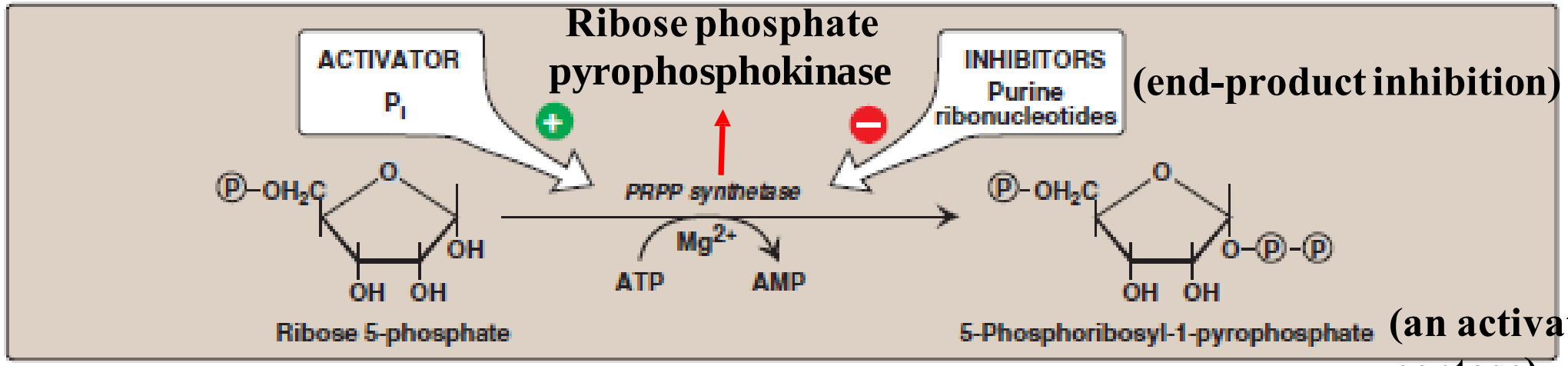
- **NOTE:**
- There are multiple sources of the atoms of nitrogenous bases, u don't have to memorize that carbon number 5 is coming from CO2....., you just need to know that there are different sources and what are these sources.

- **Glutamine** is going to donate its side chain amide group turning into glutamate.

- **Glycine**, whole of it will enter and it is going to act as a source of multiple atoms (alpha carbon, carboxyl and amino groups)

- **Aspartate**, as we learned before it is going to be added all then fumarate will be released and only amino group stays.

Synthesis of Purine Nucleotides



Steps:

1. Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

The sugar moiety of PRPP is ribose, therefore, ribonucleotides are the end products of de novo purine synthesis.

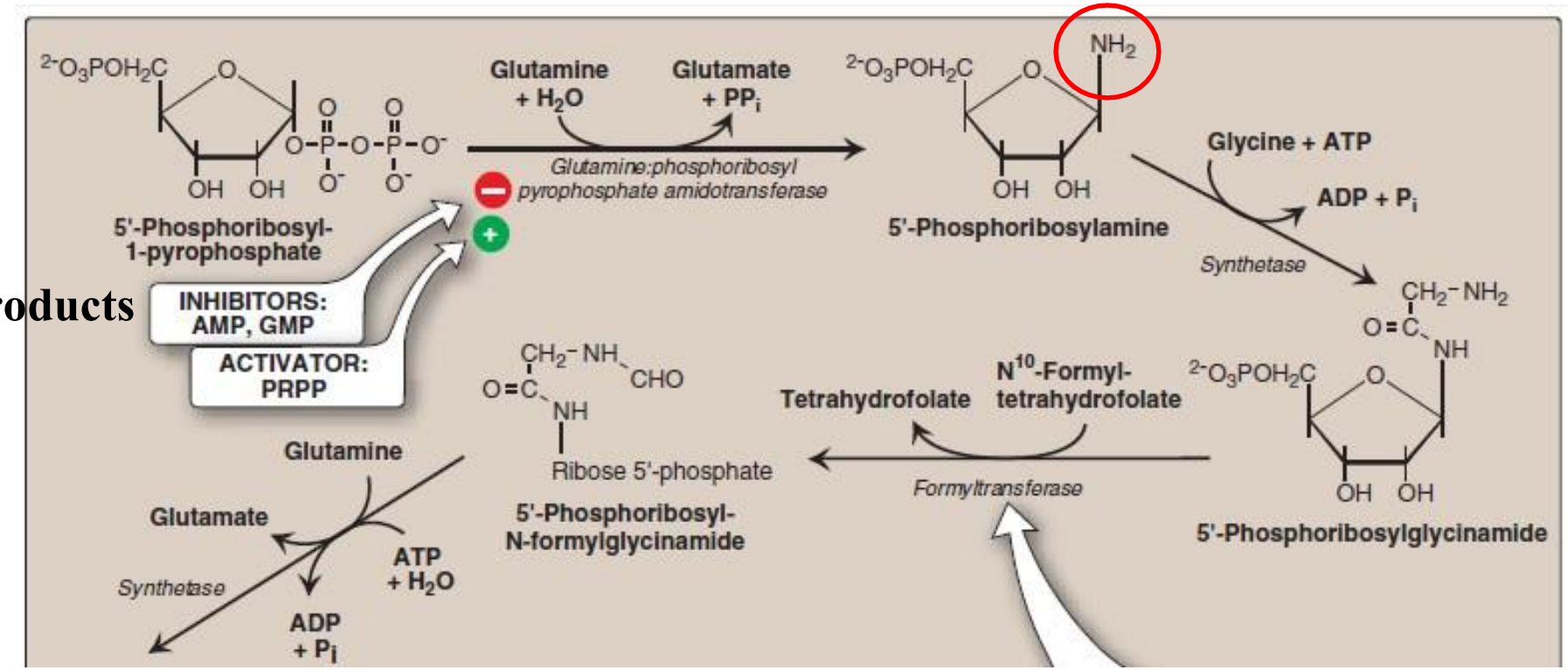
When deoxy ribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced

- **The complement in this slide:**
- What is the source of Ribose-5-phosphate? PPP (pentose phosphate pathway)
- I'm going to start with Ribose-5-phosphate, so to be able to tell whether this R5P continues in the PPP or goes to nucleotide synthesis, **we have to activate it**, how? By **adding a pyrophosphate.**
- The pyrophosphate will be added on **carbon number 1.**
- Why on carbon number one and not on the already presented phosphate? Because nitrogenous base will be added here (like marker for this site, this is the site that the synthesis of the nitrogenous base will start.
- The product is called 5-phosphoribosyl-1-pyrophosphate **(PRPP) (an activated pentose)**
- The enzyme that catalyses this reaction is called ***PRPP synthetase*** or ***Ribose phosphate pyrophosphokinase*** (the same enzyme but different name)
- PRPP is now activated so this means that we will proceed with production of nitrogenous bases (talking about purine --> **A** and **G**).

انتبه لموضوع ال regulation للخطوات المذكورة الدكتورة قالت مهم ايش بعمل inhibition وايش بعمل activation

Synthesis of Purine Nucleotides

End products



Steps:

2. Synthesis of 5'-phosphoribosylamine (the committed step in purine nucleotide biosynthesis).

3. Synthesis of inosine monophosphate, the "parent" purine nucleotide

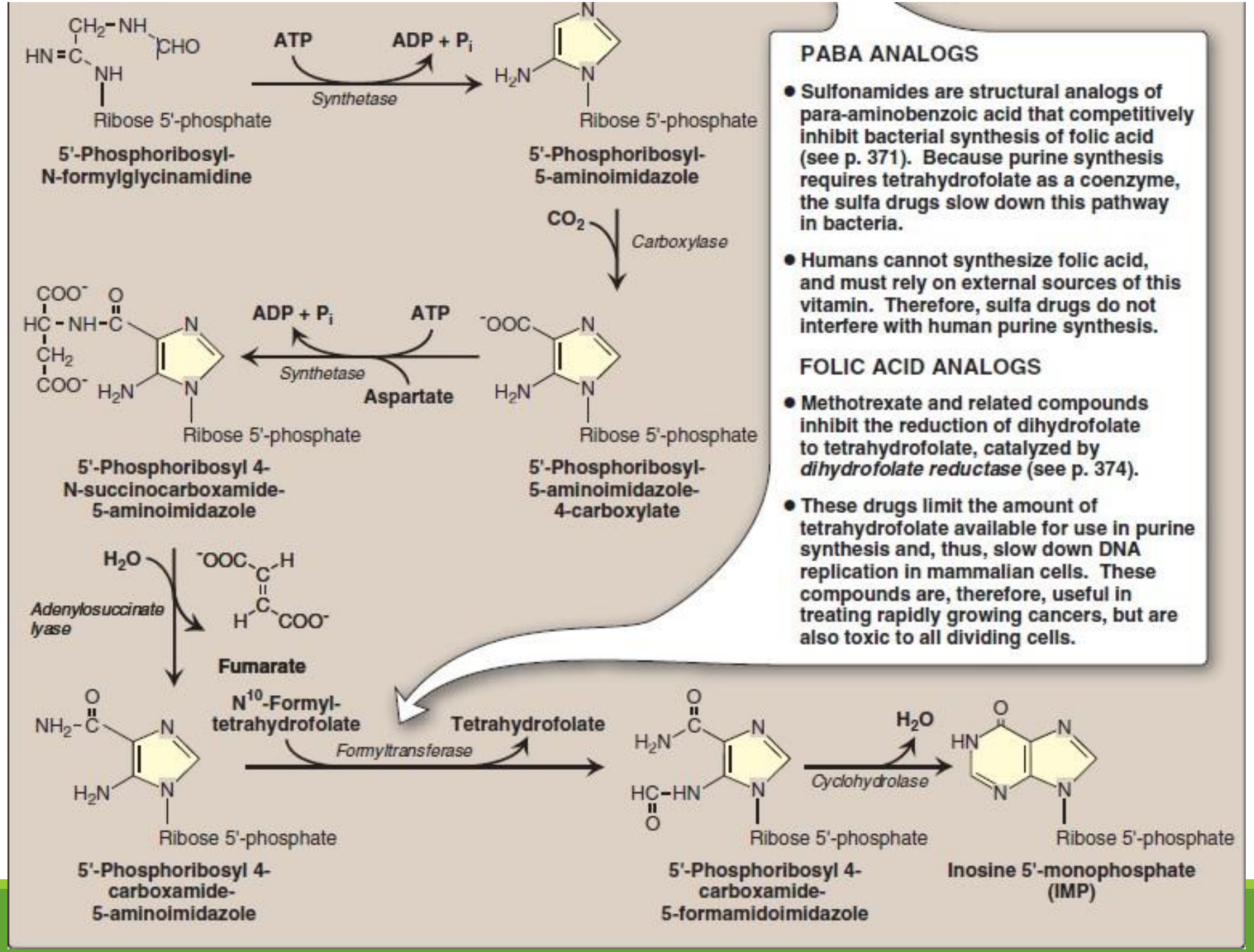
The next nine steps lead to the synthesis of IMP, whose base is hypoxanthine

This pathway requires ATP as an energy source.

- **NOTE: you don't have to memorize the exact order and names of intermediates , only the key components of the pathway.**

- **The complement in this slide:**
- 1. After the ribose activation, the pyrophosphate is replaced with an amine group derived from the side chain of **Glutamine**.
- 2. Now, **Glycine** is added, coupled with the hydrolysis of **ATP**, notice that nucleotide synthesis happens only with the presence of ATP which is available in the well fed state, so anabolic and catabolic pathways are in synergy, and all pathways are interconnected.
- 3. A formyl group is added, which was donated from N10-formyl-tetrahydrofolate.
- 4. An amine group is added again donated by glutamine, coupled with ATP hydrolysis.

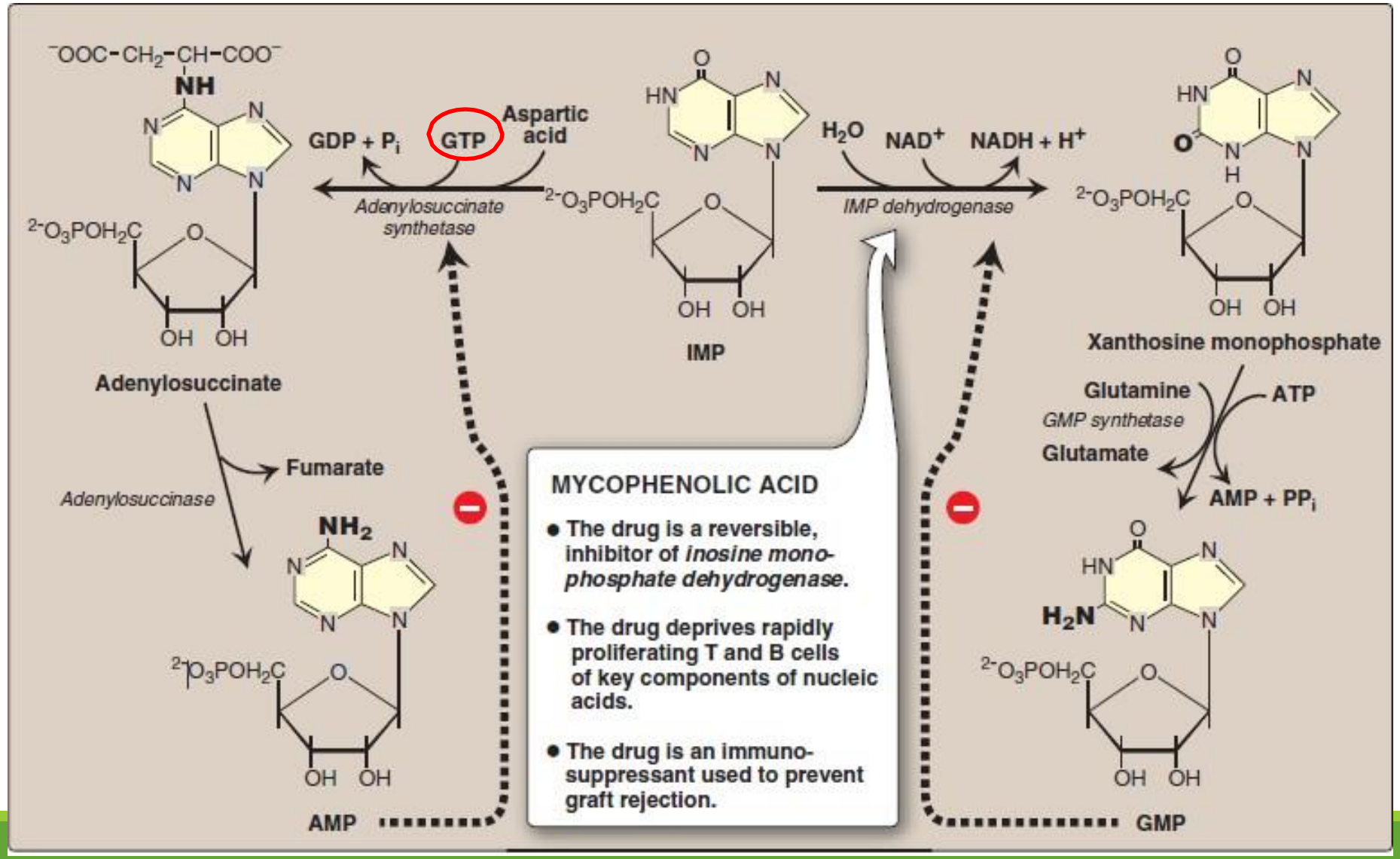
Synthesis of Purine Nucleotides



- **The complement in this slide:**

- 5. The ring is closed, coupled with ATP hydrolysis.
- 6. A carbon is added from CO_2.
- 7. Aspartate is added coupled with ATP hydrolysis.
- 8. Fumarate is removed.
- 9. A formyl group is donated again from N10-formyl- tetrahydrofolate.
- 10. The ring is closed making **Inosine 5'-monophosphate (IMP)**, which a very important molecule. (the first nucleotide that is formed but not used nether in DNA or RNA(not AMP or GMP))
- **IMP important intermediate.**

Synthesis of Purine Nucleotides
4. Conversion of IMP to AMP and

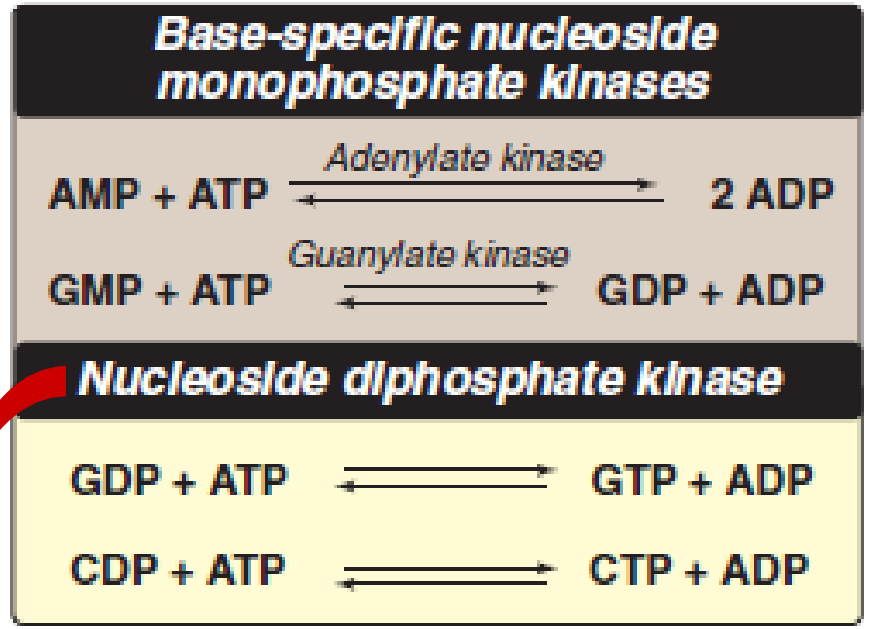


- **The complement in this slide:**

- IMP is a branching point for AMP and GMP synthesis.
- For GMP, IMP is first oxidized using *IMP dehydrogenase* with the help of NAD+ making XMP(type of nucleotide but not in DNA or RNA) , then an amine group is added using glutamine coupled with ATP hydrolysis. GMP (the final product) inhibits *IMP dehydrogenase.*
- For AMP, aspartic acid is added coupled with GTP hydrolysis using *adenylosuccinate synthetase*, then fumarate is removed, so the amine group stays making AMP, AMP inhibits *adenylosuccinate synthetase.*
- If I have alot of GMP it will inhibit its own synthesis and IMP will be converted into AMP and vice versa.

Synthesis of Purine Nucleotides

5. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates



Base-specific nucleoside monophosphate kinases do not discriminate between ribose or deoxyribose in the substrate

But it discriminate between adenine and guanine so each one has specific enzyme.

ATP is the general source of the phosphate, since it is present in higher concentrations than the other nucleoside triphosphates.

Adenylate kinase (AK) is particularly active in liver and muscle

AK maintains an equilibrium among AMP, ADP, and ATP

Broad specificity not like the monophosphate kinases بميزش بين حدا بشتغل عالكل

Synthetic inhibitors of purine synthesis

Synthetic inhibitors of purine synthesis (the sulfonamides1), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions

Other purine synthesis inhibitors, such as structural analogs of folic acid (such as, methotrexate2), are used as drugs that control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA.

Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, GI tract, immune system, or hair follicles.

Thus, anticancer drugs result in adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

- **The complement in this slide:**

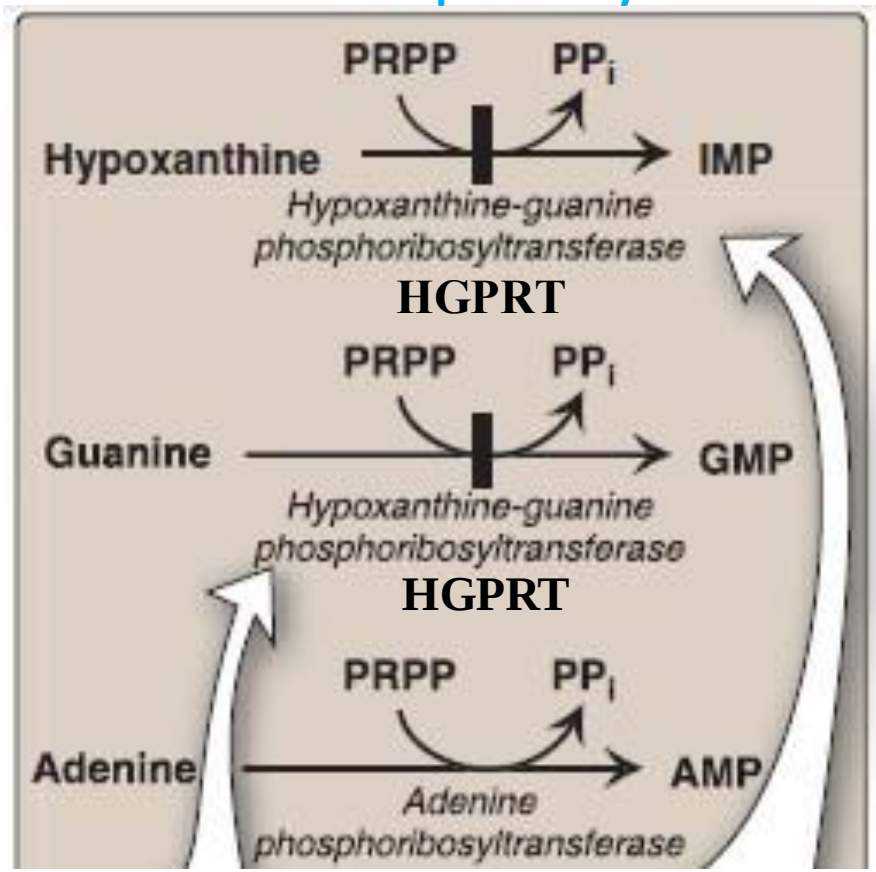
- We use synthetic purine synthesis inhibitors (as well as pyrimidine synthesis inhibitors) as anti-cancer agents, they are not specific to cancer cells, but fast growing cells in general are more affected, an example is **methotrexate** (purine and pyrimidine synthesis inhibitors).
- Some antibiotics are used as synthetic purine synthesis inhibitors specific for bacteria, like sulfonamides.

هذا النوع من المضادات ما بقتلها وانما بوقف النمو تاعها
ايش سميناه بالفارما؟
static

Salvage pathway for purines

HGPRT:Hypoxanthine-Guanine Phosphoribosyltransferase

APRT: Adenine Phosphoribosyltransferase



Salvage pathway for purines is purine synthesis from:

1. The normal turnover of cellular nucleic acids
2. Diet purines that are not degraded (small amounts)

I have the nitrogenous base already, but I need to add the sugar

Conversion of purine bases to nucleotides:

Both APRT(salvage adenine) and HGPRT(salvage guanine and Hypoxanthine) use PRPP as the source of the ribose 5-phosphate group.

If I salvage the Hypoxanthine, I will get IMP--> produce AMP and GMP

PP is released and hydrolyzed by pyrophosphatase making these reactions irreversible.

Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.

Salvage pathway for purines-Lesch-Nyhan syndrome

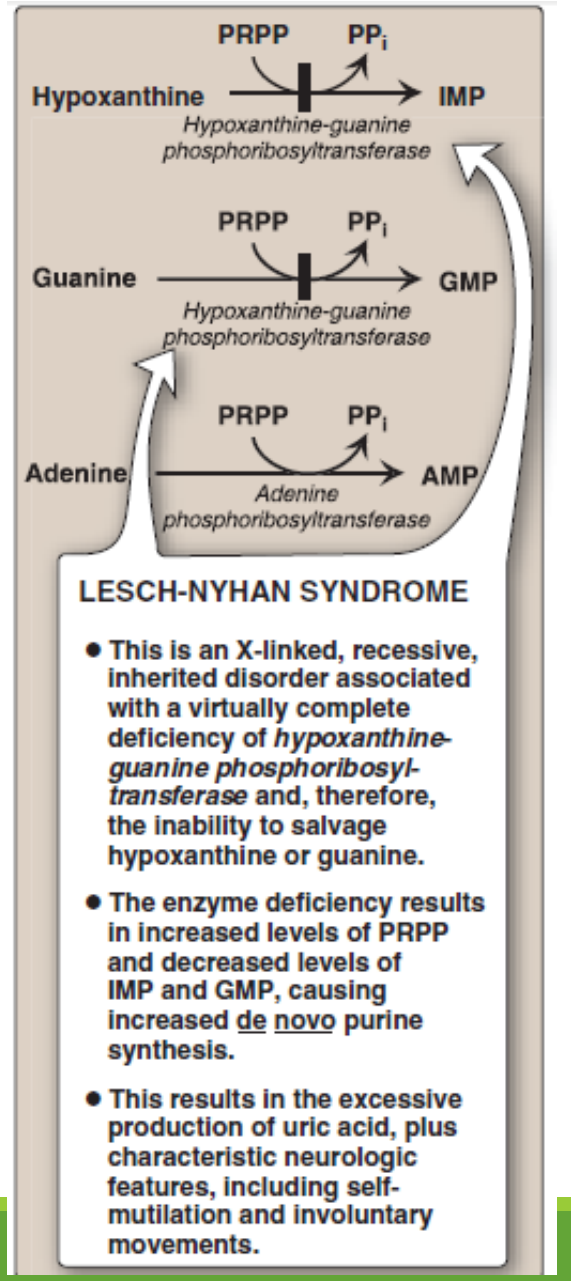
A rare, X-linked, recessive disorder associated with HGPRT deficiency.

Inability to salvage hypoxanthine or guanine resulting in high amounts of uric acid (the end product of purine degradation)

Increased PRPP levels and decreased IMP and GMP levels.
The committed step in purine synthesis has excess substrate and decreased inhibitors available, and **de novo purine synthesis is increased.**

- **NOTE:** AMP will be synthesized by de novo and salvage--> increase in the concentration of AMP-->increase the degradation--> accumulation of Uric acid

The decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid (hyperuricemia)



Lesch-Nyhan syndrome

Hyperuricemia results in:

1. Uric acid stones in the kidneys (urolithiasis)
2. The deposition of urate crystals in the joints (gouty arthritis) and soft tissues.

NOTE: uric acid accumulation is also present in gout disease, so lesch-nyhan syndrome has very similar symptoms to gout.

The syndrome is characterized by:

Motor dysfunction
Cognitive deficits
Behavioral disturbances that include self-mutilation (biting of lips and fingers)

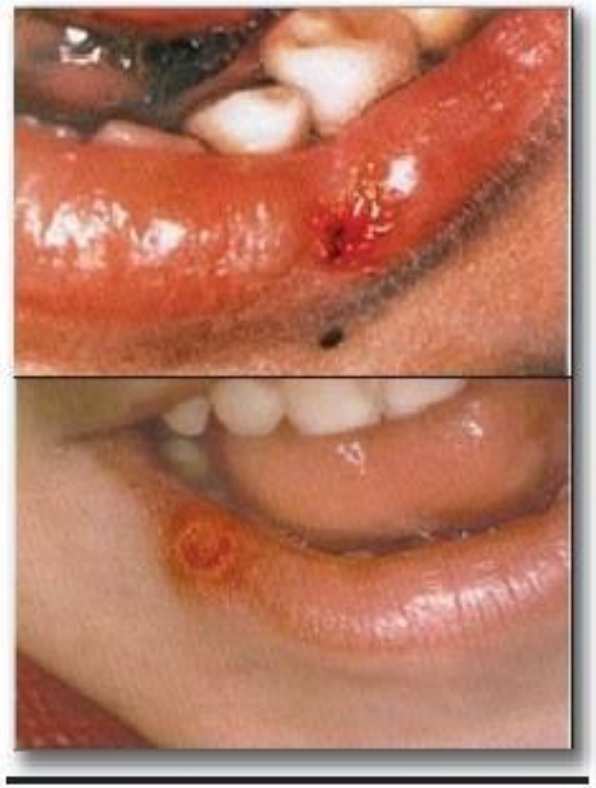
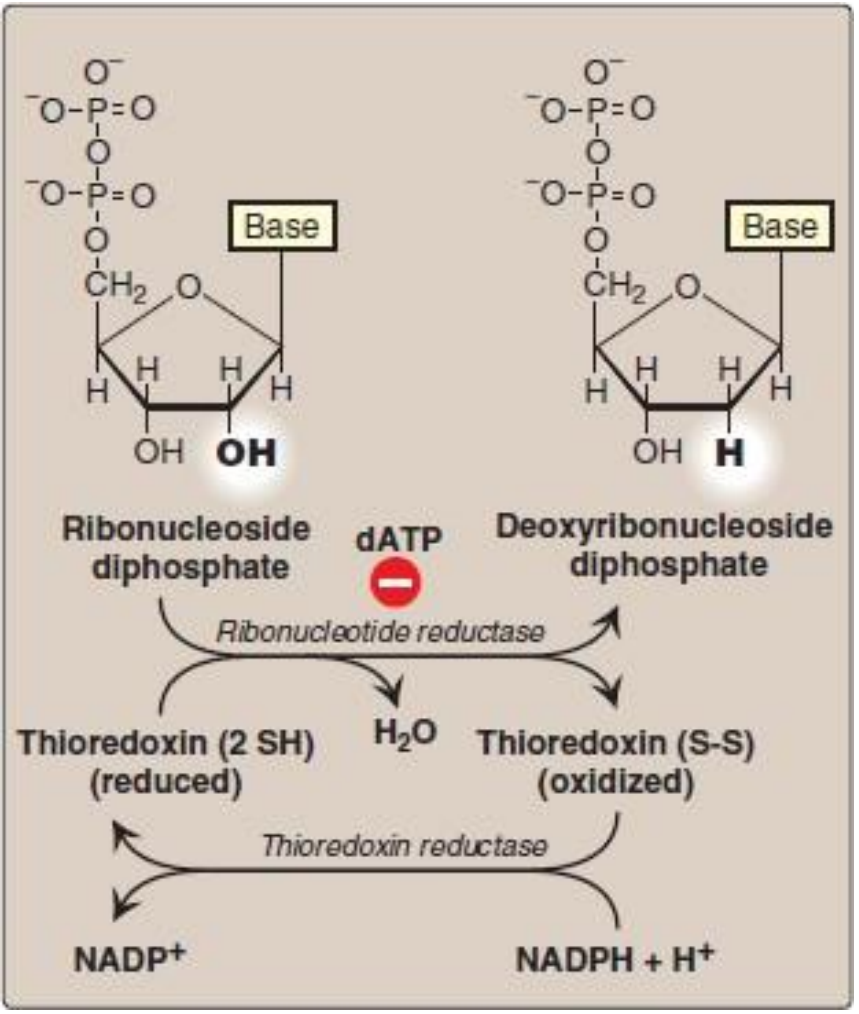


Figure 22.11
Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.

Synthesis of Deoxyribonucleotides



2'-deoxyribonucleotides are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle

The only reason to synthesis 2'-deoxyribonucleotides is for DNA replication.

The same enzyme acts on pyrimidine ribonucleotides

1. Ribonucleotide reductase (RR)

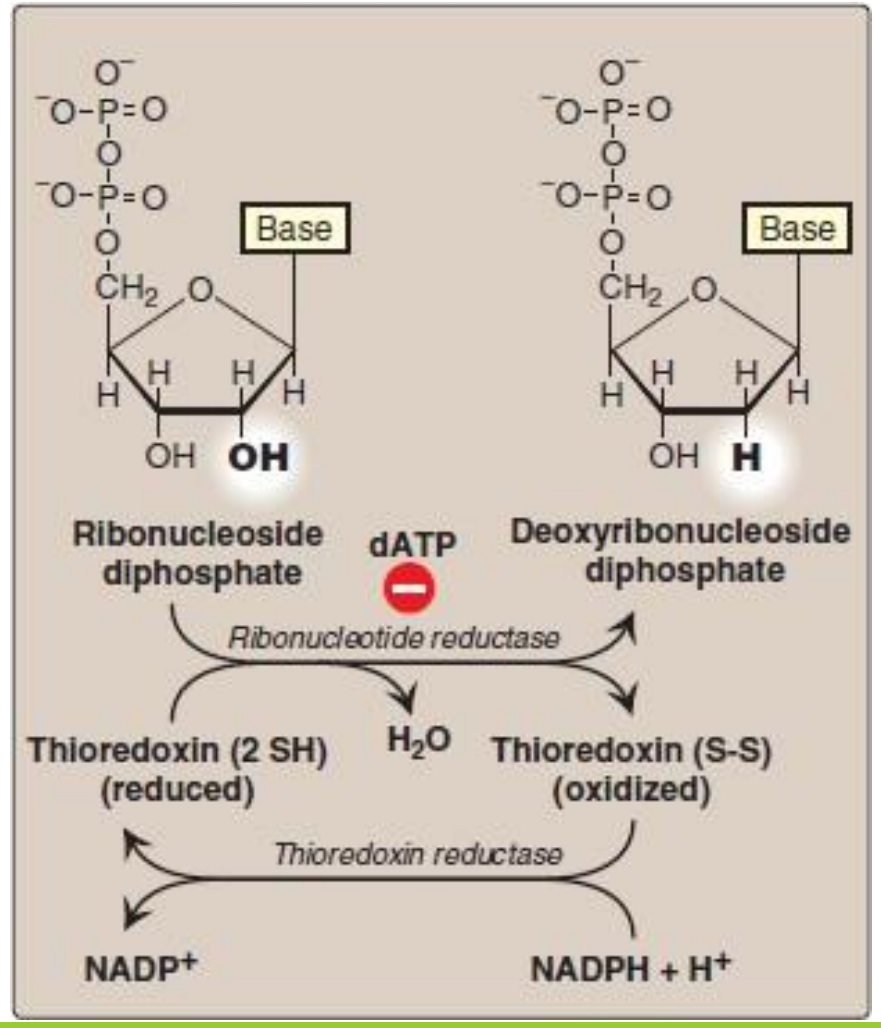
- **NOTE: RR only acts on nucleoside diphosphates, (ADP, GDP, etc.**

RR is specific for the reduction of:

A. Purine nucleoside diphosphates (ADP and GDP) to their deoxyforms (dADP and dGDP).

B. Pyrimidine nucleoside diphosphates, cytidine diphosphate (CDP) and uridine diphosphate (UDP) to their deoxyforms (dCDP, and dUDP).

Synthesis of Deoxyribonucleotides



NOTE: This reaction uses thioredoxin (reduced form 2 SH) as a reducing agent to remove the oxygen (reduce) the nucleotide, this reaction oxidizes thioredoxin to the oxidized form (S-S), so it has to be recycled back to the reduced form using ***thioredoxin reductase*** with the help of NADPH as a coenzyme. Synthesis happen in well fed state

2. Regeneration of reduced enzyme:

Thioredoxin—a peptide coenzyme of RR

3. Regeneration of reduced thioredoxin:

Thioredoxin must be converted back to its reduced form

NADPH + H+ are needed

The reaction is catalyzed by thioredoxin reductase

Regulation of deoxyribonucleotide synthesis

Ribonucleotide reductase is composed of two non identical dimeric subunits, R1 and R2

RR is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis.

1. Activity sites (allosteric sites):

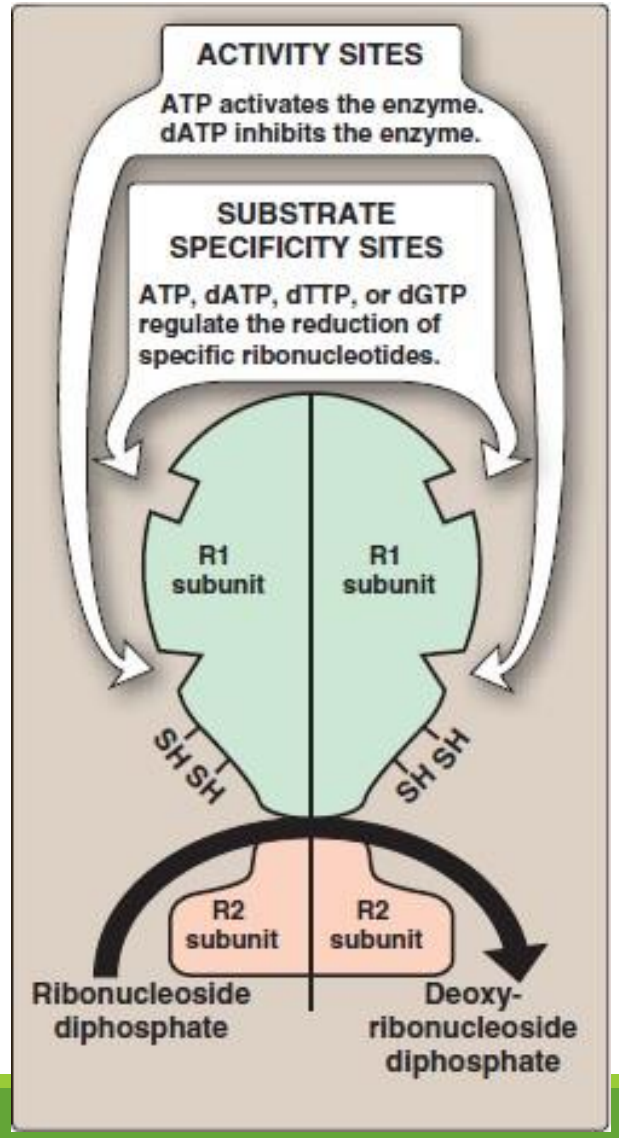
a. dATP inhibits the enzyme and prevents the reduction of any of the four nucleoside diphosphates resulting in preventing DNA synthesis.

b. ATP activates the enzyme.

2. Substrate specificity sites (allosteric sites):

Nucleoside triphosphates regulate substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides.

dTTP binding activates the reduction of GDP to dGDP at the catalytic site.



- **The complement in this slide:**

- RR has two types of regulation.
- The first type is activity regulation, which is regulated by the **activity site** on the enzyme, ATP allosterically activates the reaction and dATP allosterically inhibits the reaction.
- The other type of regulation is the specificity type, which regulates and guarantees the balance between the amounts of different deoxy nucleotides, and it is regulated by the **substrate specificity site**, so if we have too much of one deoxy nucleotide (ex. dTTP), the production of another deoxy nucleotide is stimulated (ex. GDP to dGDP).

Hydroxyurea and ribonucleotide reductase

The drug hydroxyurea destroys the free radical required for the activity of ribonucleotide reductase

Hydroxyurea inhibits the generation of substrates for DNA

synthesis. Hydroxyurea has been used in the treatment of cancers

such as CML

NOTE: notice that this drug does not inhibit the synthesis of nucleotides, but only the making of deoxy nucleotides, so it only affects DNA synthesis, which is associated with cell replication.

V2: SLIDE 14 and 20

synthetase **instead of synthase**